TOXICOLOGICAL AND BIOCHEMICAL STUDIES ON SOME TRIALKYLGERMANIUM COMPOUNDS

BY

JILL E. CREMER and W. N. ALDRIDGE

*From the Toxicology Research Unit, Medical Research Council Laboratories,
Carshalton, Surrey*

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The toxicity of triethyl- and tri-*n*-butyl-germanium acetates has been studied after their administration to rats. Both compounds had a low toxicity. Triethylgermanium had less than one-tenth of the toxicity of triethyltin or triethyl-lead and, unlike them, it did not appear to have a predominant action on the central nervous system.

In biochemical studies *in vitro*, tri-*n*-butylgermanium was found to be more active than trimethyl-, triethyl- or tri-*n*-propyl-germanium in inhibiting both glucose oxidation by slices of rat brain cortex and processes involved in oxidative phosphorylation by rat liver mitochondria. All the germanium compounds tested had less than one-hundredth the activity of the corresponding trialkyltin and trialkyl-lead compounds.

The toxicity and biochemical actions of a series of trialkyltin and trialkyl-lead compounds have been studied in considerable detail in this laboratory over the past few years. In both groups of compounds the lower members of the series have been shown to be toxic to animals with a predominant action on the central nervous system. Detailed symptomatological descriptions of the action of trialkyltins on rats and rabbits have been given by Barnes and Stoner (1958), Stoner, Barnes, and Duff (1955), and Magee, Stoner, and Barnes (1957), and of trialkyl-leads by Cremer (1959) and Cremer and Callaway (1961). Biochemical studies have mainly been concerned with the action of trialkyltin and trialkyl-lead compounds on glucose metabolism by rat brain (Cremer, 1957, 1959, 1961, 1962; Aldridge, Cremer, and Threlfall, 1962) and on processes involved in oxidative phosphorylation by mitochondria isolated from rat liver (Aldridge, 1958; Aldridge *et al.*, 1962).

We now wish to report some similar data for a series of trialkylgermanium compounds. Unlike the trialkyltin and trialkyl-lead compounds, trialkyl-germanium compounds have a low toxicity to rats and are relatively inert in the biochemical systems we have studied. Although the results were largely negative, their publication seemed warranted since possible commercial uses of trialkylgermanium compounds are under investigation. They indicate

that the toxicological hazards to man from the use of trialkylgermanium would be less than those from the corresponding tin and lead compounds.

Materials and Methods

Pure samples of the germanium compounds were supplied by Dr. G. M. van der Want of the Institute for Organic Chemistry T.N.O., Utrecht, Holland.

Adult albino rats of 175-230 g. body weight were used.

Administration of Germanium Compounds in Animals.—Triethylgermanium acetate was given to rats either intravenously (tail vein) as a solution in ethanol (0.05 ml. per rat) or orally by stomach tube as a solution in arachis oil. Tri-*n*-butylgermanium acetate was given to rats orally as a solution in arachis oil followed nine days later by an intraperitoneal injection as a solution in dimethylformamide. (Each rat received not more than 0.1 ml. dimethylformamide.)

Mitochondria.—Mitochondria were isolated from rat liver by the procedure previously described (Aldridge, 1958). Respiration of the mitochondria was measured in the presence and absence of apyrase (Aldridge and Stoner, 1960). Details are given in the footnote to Table 2. The adenosine-triphosphatase (ATP-ase) activity of the mitochondria was measured in the presence and absence of 30 μ M 2,4-dinitrophenol (DNP), as previously described by Aldridge (1958).

TABLE 1
TOXICITY OF TRIETHYL- AND TRI-N-BUTYL-GERMANIUM TO MALE RATS

Compound	No. of Animals	Dose (mg./kg.)	Route	Reaction	Death	Time of Death after Injection
Triethylgermanium acetate	5	50	I.V.	1 rat immediately after the injection had violent convulsions, collapsed and died. Surviving rats initially showed rapid breathing and remained in a stretched out position for 2 hr. By 18 hr, recovery was complete	1	1 minute
	4	31.25	Oral	No signs of poisoning		
	4	62.5	Oral	Slight loss of weight during first week, thereafter regained weight		
	4	125	Oral	Loss of weight during first week, thereafter regained weight		
	4	250	Oral	No obvious cause of death. Liver very dark and lungs haemorrhagic	3	1-2 hours
Tri-n-butylgermanium acetate	4	111 after 9 days	Oral	Some weight loss after I.P. injection		
		250	I.P.			
	4	167	Oral	No signs of poisoning		
	4	250	Oral	Some weight loss after I.P. injection		
		125 after 9 days	I.P.			
	4	375	Oral	No signs of poisoning		

Brain Slices.—Rat brain cortex slices were prepared and used as described by Cremer (1957). The details are given in the footnote to Table 4.

Results

Toxicity to Rats.—Only triethyl- and tri-n-butylgermanium were tested. Summaries of the response of the rats are given in Table 1. After the oral administration of triethylgermanium acetate three out of a group of four rats receiving the highest dose died within two hours. Macroscopical immediate post-mortem examination revealed that severe haemorrhage had occurred throughout the lungs, the liver was a deep purple colour, and the right auricle was distended. The intestine, spleen, and kidneys appeared normal. The surviving rat and those receiving 125 mg./kg. body weight did not gain weight during the first five days after the oral administration, but thereafter they showed a normal weight gain and no other signs of poisoning were detectable.

From the present results it is not possible to determine the LD_{50} to rats of triethylgermanium but it is probably between 125 and 250 mg./kg. body weight. This compound has therefore at least less than one-tenth of the toxicity of triethyltin or triethyl-lead.

Tri-n-butylgermanium acetate was administered orally at four different dose levels to groups of rats each containing four animals. No signs of poisoning were observed. Nine days after the oral dose an intraperitoneal injection of tri-n-butylgermanium acetate was given to animals in two of the four groups as indicated in Table 1. In both of these groups the animals showed some loss of weight but no other signs of poisoning were observed. Tri-n-

butylgermanium acetate has a low toxicity to rats.

Mitochondrial Respiration.—Previous work using liver mitochondria had shown that when pyruvate was added as a substrate (with a small amount of 'sparker' fumarate) the rate of oxygen uptake could be increased three- to four-fold by the addition of potato apyrase (Aldridge, 1957). This additional oxygen uptake must therefore be associated with the phosphorylation of adenine nucleotide. Furthermore, this oxygen uptake induced by apyrase was inhibited by low concentrations of trialkyltin (Aldridge, 1958) and trialkyl-lead compounds (Aldridge *et al.*, 1962).

The effect of a series of trialkylgermaniums on unstimulated and apyrase-stimulated respiration of rat liver mitochondria is shown in Table 2. Tri-

TABLE 2
EFFECT OF TRIALKYLGERMANIUM COMPOUNDS ON RESPIRATION OF RAT LIVER MITOCHONDRIA

Trialkylgermanium	Molar Concentration	Oxygen Uptake (% of control)	
		No Apyrase	With Apyrase
Methyl	10^{-3}	106	93
	10^{-4}	96	101
	10^{-5}	105	94
Ethyl	10^{-3}	107	61
	10^{-4}	122	75
	10^{-5}	25	0
n-Propyl	10^{-4}		
	10^{-5}	103	86

Each flask contained 3 ml. of a solution of pH 6.8 containing adenosine 5-phosphate (1.15 mM), ATP (1.06 mM), KCl (0.1 M), $MgCl_2$ (14 mM), ethylenediaminetetra-acetic acid (1.0 mM), potassium phosphate (15 mM), sucrose (30 mM), pyruvate (10 mM), fumarate (1.0 mM) and 0.3 ml. of mitochondrial suspension equivalent to 0.15 g. wet weight of original liver. Where indicated, sufficient apyrase to hydrolyse 800 μ g. atom P/hr. was added. Trialkylgermanium compounds were added as 0.03 ml. samples in dimethylformamide. Incubation was at 37°C. Oxygen uptake was measured at 5-min. intervals from 10 to 40 min. QO_2 values (μ l. O_2 /mg. protein/hr.) were 33 for the unstimulated control and 100 for the control stimulated with apyrase.

methyl- was the least and tri-n-butylgermanium the most active, the latter compound showing some inhibition of apyrase-stimulated respiration at a concentration of 10^{-5} M. The trialkylgermanium compounds were less active than the trialkyltin or trialkyl-lead compounds, which produced 50% inhibition of apyrase-stimulated respiration at concentrations of 10^{-6} M or less.

Mitochondrial Adenosine Triphosphatase.—The addition of DNP (30 μ M) to liver mitochondria brings about an approximately 20-fold increase in ATP-ase activity. Aldridge (1958) has shown that this increase is not affected by respiratory inhibitors, such as cyanide and phenylarsenious acid, but it is inhibited by low concentrations of trialkyltin and trialkyl-lead compounds. From this and other evidence it has been suggested that trialkyltins and trialkyl-leads inhibit a step in the energy-transferring chain between electron transport and the formation of adenosine triphosphate. The effect of trialkylgermaniums on mitochondrial ATP-ase activity is shown in Table 3. Trimethylgermanium acetate at high concentrations inhibited unstimulated ATP-ase activity but had no effect on the ATP-ase activity stimulated by DNP. Triethyl-, tri-n-propyl- and tri-n-butyl-germanium compounds resembled the trialkyltin and trialkyl-lead compounds inasmuch

as at the higher concentrations they increased the ATP-ase activity of the mitochondria but inhibited the stimulation of ATP-ase by DNP. These results suggest that these three trialkylgermanium compounds do have an inhibitory effect on oxidative phosphorylation processes but that the concentrations required are at least one hundred times greater than for the corresponding trialkyltin and trialkyl-lead compounds.

When high concentrations of compounds such as the trialkylgermaniums, which are sparingly water soluble, are used, it is very likely that they will cause structural damage to the mitochondria. It is known that damage to intact mitochondria releases latent ATP-ase activity. Gross swelling of the mitochondria was indeed seen when high concentrations of tri-n-propyl- and tri-n-butyl-germaniums were used (Table 3).

Slices of Rat Brain Cortex.—Previous studies on the respiration of rat tissues have shown that the

TABLE 3
EFFECT OF TRIALKYLGERMANIUM COMPOUNDS
ON ADENOSINE TRIPHOSPHATASE ACTIVITY OF RAT
LIVER MITOCHONDRIA

Trialkylgermanium	Molar Concentration	Adenosine Triphosphatase Activity (% of control)	
		No DNP	With DNP
Methyl	10^{-3}	40	85
	10^{-4}	60	102
	10^{-5}	87	102
Ethyl	10^{-6}	100	102
	10^{-3}	514	47
	10^{-4}	230	94
n-Propyl	10^{-5}	100	100
	10^{-6}	100	100
	10^{-3}	1004*	59*
n-Butyl	10^{-4}	302	37
	10^{-5}	126	82
	10^{-6}	100	100
	10^{-3}	490*	70*
	10^{-4}	293*	27*
	10^{-5}	127	38
	10^{-6}	100	89
	10^{-7}	106	100

*The germanium compound was out of solution, and a clearing of the mitochondria within 1 min. indicated gross swelling.

Each beaker contained 3 ml. of a solution of pH 6.8 containing ATP (3 mM), KCl (0.1 M), $MgCl_2$ (14 mM), EDTA (1.0 mM), glycylglycine (16.7 mM), and sucrose (30 mM). Where indicated, DNP (30 μ M) and trialkylgermanium compounds in 0.03 ml. dimethylformamide were added. The beakers were shaken at 37°C. Liberation of inorganic phosphate was determined 10 min. after the addition of mitochondria. Control activity was approx. 0.5 μ g. atom P/mg. protein/hr. and this was stimulated by 2:4 dinitrophenol (30 μ M) to approx. 10 μ g. atom P/mg. protein/hr.

TABLE 4
EFFECT OF TRIALKYLGERMANIUM
COMPOUNDS ON THE METABOLISM OF SLICES
OF RAT BRAIN CORTEX

Trialkylgermanium	Molar Concentration	% of Control		
		QO_2 *	Lactic Acid	Pyruvic Acid
Methyl	1×10^{-4}	103	—	—
	3×10^{-5}	103	—	—
Ethyl	5×10^{-4}	35	485	39
	1×10^{-4}	66	565	71
	3×10^{-5}	110	250	100
n-Propyl	1×10^{-4}	42	410	40
	3×10^{-5}	95	115	100
n-Butyl	3×10^{-4}	42	214	34
	2×10^{-5}	70	280	55
	5×10^{-6}	89	100	82
	2×10^{-6}	100	100	93

*Oxygen uptake

Each flask contained 3.0 ml. of Kreb's Ringer phosphate solution with glucose 0.01 M, tissue slice 50-60 mg. wet weight, 0.03 ml. of trialkylgermanium compound in dimethylformamide as indicated, and 0.2 ml. of 20% (w/v) KOH in the centre-well for absorption of CO_2 . The gas phase was 100% O_2 . Incubation was at 37°C. for 75 minutes. QO_2 was calculated as μ l. of O_2 /mg. dry weight of tissue/hour. The control QO_2 value was 13.5. The control values for lactic and pyruvic acid were 180 μ g. and 10.0 μ g. respectively, estimated as the amount present in the medium after 75 minutes' incubation.

oxidation of glucose by brain slices is particularly sensitive to low concentrations of trialkyltin and trialkyl-lead compounds (Cremer, 1957; Aldridge *et al.*, 1962). Both groups of compounds inhibited oxygen uptake, lowered the level of pyruvic acid, and increased the level of lactic acid. It can be seen from the results given in Table 4 that tri-ethyl-, n-propyl- and n-butyl-germanium acetates inhibited the oxidation of glucose by slices of brain cortex in the manner described above for the corresponding tin and lead compounds. The main difference was

that the concentrations of the germanium compounds required for inhibition were at least one hundred times greater.

Conclusion

The results of experiments reported in this paper show that the action on certain biological systems of the trialkylgermanium compounds was similar to that of the trialkyltin and trialkyl-lead compounds, but effective concentrations were over one hundred times greater. The low activity found in biochemical studies *in vitro* was reflected in the low toxicity to rats. No biological properties peculiar to the trialkylgermaniums were detected and they do not appear to have a predominant action on the central nervous system.

It is of intrinsic interest that the trialkylgermanium compounds have such a relatively low biological activity compared with their analogues of tin and lead since the three metals are in the same subgroup, IVB, of the Periodic Table. Increasing molecular weight of $\text{Ge} < \text{Sn} < \text{Pb}$ is accompanied by increasing basicity. Trialkyltins and trialkyl-leads

form coloured complexes with dithizone (Aldridge and Cremer, 1957; Cremer, 1959) but the trialkylgermaniums do not. Whether or not ionic character is an essential prerequisite for biological activity in this series of trialkyl-metals remains to be determined.

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